Generation of an iPSC line from a patient with tyrosine hydroxylase (TH) deficiency: TH-1 iPSC

Jung-Klawitter, Sabine ; Blau, Nenad ; Sebe, Attila ; Ebersold, Juliane ; Göhring, Gudrun ; Opladen, Thomas

Abstract: Fibroblasts from a male patient with compound heterozygous variants in the tyrosine hydroxylase gene (TH; OMIM: 191290; c.[385-C>T]; [692-G>C]/p.[R129*]; [R231P]), the rate-limiting enzyme for dopamine synthesis, were reprogrammed to iPSCs using episomal reprogramming delivering the reprogramming factors Oct3/4, Sox2, L-Myc, Lin28, Klf4 and p53 shRNA Okita et al. (2011). Pluripotency of TH-1 iPSC was verified by immunohistochemistry and RT-PCR analysis. Cells exhibited a normal karyotype and differentiated spontaneously into the 3 germ layers in vitro. TH-1 iPSC represents the first model system to study the pathomechanism of this rare metabolic disease and provides a useful tool for drug testing.

DOI: https://doi.org/10.1016/j.scr.2016.10.008

Originally published at:
Jung-Klawitter, Sabine; Blau, Nenad; Sebe, Attila; Ebersold, Juliane; Göhring, Gudrun; Opladen, Thomas (2016). Generation of an iPSC line from a patient with tyrosine hydroxylase (TH) deficiency: TH-1 iPSC. Stem Cell Research, 17(3):580-583.
DOI: https://doi.org/10.1016/j.scr.2016.10.008

Stem Cell Research 17 (2016) 580–583

Contents lists available at ScienceDirect

Stem Cell Research

journal homepage: www.elsevier.com/locate/scr

Lab Resource: Stem Cell Line

Generation of an iPSC line from a patient with tyrosine hydroxylase (TH) deficiency: TH-1 iPSC

Sabine Jung-Klawitter [a,*], Nenad Blau [a], Attila Sebe [b], Juliane Ebersold [c], Gudrun Göhring [c], Thomas Opladen [a]

[a] Department of General Pediatrics, Division of Neuropediatrics and Metabolic Medicine, University Hospital Heidelberg, Heidelberg, Germany
[b] Paul-Ehrlich Institute, Langen, Germany
[c] Department of Human Genetics, Hannover Medical School (MHH), Hannover, Germany

ARTICLE INFO

Article history:
Received 7 October 2016
Accepted 18 October 2016
Available online 26 October 2016

ABSTRACT

Fibroblasts from a male patient with compound heterozygous variants in the tyrosine hydroxylase gene (TH; OMIM: 191290; c.[385-C>T]; [692-G>C]/p.[R129*]; [R231P]), the rate-limiting enzyme for dopamine synthesis, were reprogrammed to iPSCs using episomal reprogramming delivering the reprogramming factors *Oct3/4*, *Sox2*, *L-Myc*, *Lin28*, *Klf4* and *p53 shRNA* Okita et al. (2011). Pluripotency of TH-1 iPSC was verified by immunohistochemistry and RT-PCR analysis. Cells exhibited a normal karyotype and differentiated spontaneously into the 3 germ layers *in vitro*. TH-1 iPSC represents the first model system to study the pathomechanism of this rare metabolic disease and provides a useful tool for drug testing.

1. Resource table

Name of Stem Cell line	TH-1 iPSC
Institution	Department of General Pediatrics, Division of Neuropediatrics and Metabolic Medicine, University Hospital Heidelberg, Heidelberg, Germany
Person who created resource	Sabine Jung-Klawitter
Contact person and email	Sabine Jung-Klawitter; Sabine.Jung-Klawitter@med.uni-heidelberg.de
Date archived/stock date	January 2016
Origin	human fibroblasts
Type of resource	Human induced pluripotent stem cell (iPSC); generated from a male patient with compound heterozygous variants in the TH gene (NG_008128.1; OMIM: 191290; Gene ID: 7054; c.[385-C>T]; [692-G>C]/p.[R129*]; [R231P]); manually picked single clone
Sub-type	Induced Pluripotent Stem Cells (iPSCs)
Key transcription factors	Oct3/4, Sox2, L-Myc, hKlf4, Lin28, p53 shRNA (Okita et al., 2011)
Authentication	Identity and purity of cell line confirmed as shown in Fig. 1
Link to related literature	
Information in public databases	OMIM: 191290; HGNC ID: HGNC: 11782; Gene ID: 7054
Ethics	Institutional ethics committee approval obtained (No. 2016–02-04 ZB 52315) / Patient written informed consent obtained

2. Resource details

Fibroblasts from a male patient with compound heterozygous variants in the tyrosine hydroxylase gene (TH, DYT14; OMIM: 191290; HGNC: 11782; c.[385-C>T]; [692-G>C]/p.[R129*]; [R231P]) were episomally reprogrammed using the reprogramming factors Oct3/4, Sox2, L-Myc, Klf4, Lin28 and an shRNA directed against p53 according to Okita et al. (2011). Karyotype was determined by fluorescence R-banding and provided a normal diploid 46,XY karyotype (Fig. 1A). Absence of episomal plasmids was analysed by RT-PCR using EBNA-and plasmid-specific primers (Fig. 1B; Table 1). Presence of the pathogenic variants was verified by PCR followed by Sanger Sequencing (Fig. 1C, Table 1). Expression of pluripotency markers was detected by immunofluorescence staining specific for Oct3/4, Sox2, Nanog, Lin28 and SSEA-4 (Fig. 1D) as well as by RT-PCR using gene-specific primers (Fig. 1E; Table 1). Differentiation potential was tested using embryoid body (EB) formation followed by RT-PCR (Haase et al., 2009) amplifying AFP (endoderm), MLC2A (mesoderm) and βIII-tubulin (ectoderm; Fig. 1F) and immunofluorescence staining for endodermal (AFP), mesodermal (SMA) and ectodermal (βIII-tubulin) markers (Fig. 1G).

* Corresponding author at: Department of General Pediatrics, Division of Neuropediatrics and Metabolic Medicine, University Hospital Heidelberg, Im Neuenheimer Feld 669, 69120 Heidelberg, Germany.
E-mail address: sabine.jung-klawitter@med.uni-heidelberg.de (S. Jung-Klawitter).

http://dx.doi.org/10.1016/j.scr.2016.10.008

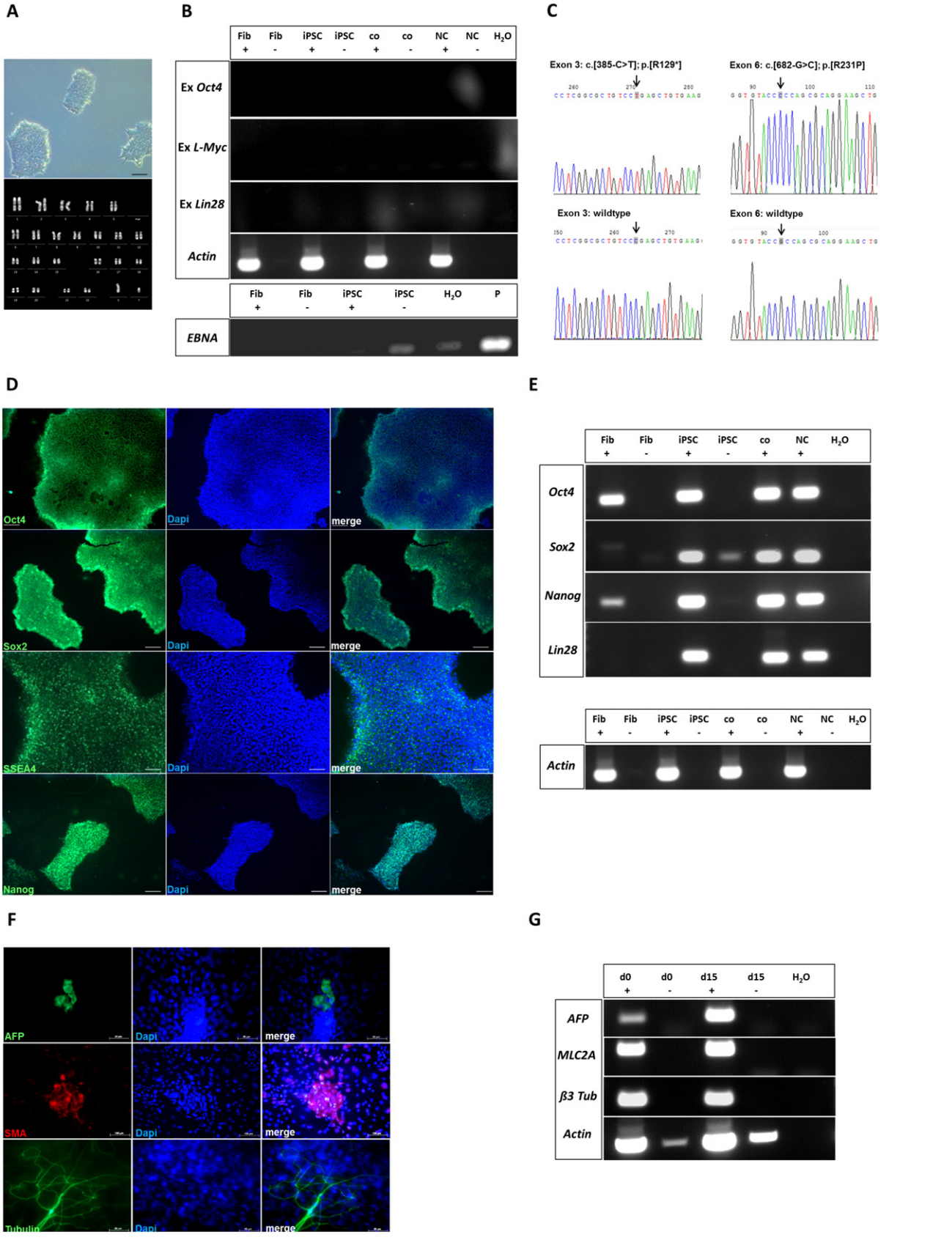

Fig. 1. TH-1 iPSCs exhibit a normal karyotype (46,XY; A). No expression from the episomal plasmids used for reprogramming was detectable with RT-PCR neither in the untransfected fibroblasts of the patient (Fib) nor in the TH-1 iPSCs (iPSC; B). Plasmid pCXLE-hOCT3/4-shp53-F (P) was used as positive control for the amplification of the EBNA site (B). Presence of both single nucleotide exchanges described for the patient was verified by PCR amplification and subsequent Sanger Sequencing (C). TH-1 iPSCs show expression of pluripotency marker genes in the immunofluorescence staining (D) as well as on RNA level as detected by RT-PCR (E). TH-1 iPSCs can be differentiated into all three germ layers using EB formation as shown by immunofluorescence staining (F) and RT-PCR (G). *Abbreviations:* +: cDNA synthesis performed with reverse transcriptase; −: cDNA synthesis performed without reverse transcriptase; P: plasmid pCXLE-hOCT3/4-shp53-F; co: control iPSC line; NC: NCCIT cells used as positive control. Scale bars represent 100 μm.

Table 1
Primers used in the study.

Gene symbol	Sequence (fwd and rvs; 5′–3′)	Product size (bp)	Annealing temperature (°C)
β-actin	CATGGAGAAAATCTGGCACCAC GCACAGCTTCTCCTTAATGTCAC	409	56
Oct3/4	GAACCAGTATCGAGAACCG TCAGTTTGAATGCATGGGAG	383	56
Sox2	CACATGTCCCAGCACTACCAG CACATGTGTGAGAGGGGCAG	77	56
Nanog	AAACAGAAGACCAGAACTGTG CAGTTGTTTTTCTGCCACCTCT	191	56
Lin28	CCATATGGTAGCCTCATGTC CAATTCTGTGCCTCCGGG	126	56
βIII tubulin	CAACAGCACGGCCATCCAGG CTTGGGGCCCTGGGCCTCCGA	242	66
AFP	ACTCCAGTAAACCCTGGTGTTG GAAATCTGCAATGACAGCCTCA	255	55
MLC2A	GAGGAGAATGGCCAGCAGGAA GCGAACATCTGCTCCACCTCA	446	60
EBNA	GGATCACTAGGTGATATCGAGC ACCAGACAAGAGTTTAAGAGATATGTATC	61	58
Oct4 (exogen)	CATTCAAACTGAGGTAAGGG TAGCGTAAAAGGAGCAACATAG	124	60
L-Myc (exogen)	GGCTGAGAAGAGGATGGCTAC TTTGTTTGACAGGAGCGACAAT	122	60
Lin28 (exogen)	AGCCATATGGTAGCCTCATGTCCGC TAGCGTAAAAGGAGCAACATAG	251	60
TH Exon 3	CCGCGGTTCATTGGGCG CACCAGCTCACCTCAAACAC	230	56
TH Exon 6	GGCTTCTCGGACCAGG GACAAGATGGGTCCTCC	170	57

3. Materials and methods

3.1. iPSC reprogramming

Fibroblasts were isolated and cultured in DMEM supplemented with 10% FBS, 1% Penicillin/Streptomycin, 0.1 mM non-essential amino acids and 50 μM β-mercaptoethanol (Invitrogen). Cells were reprogrammed in passage 13 by electroporation using the 4D-Nucleofector System (Lonza) and plasmids pCXLE-hOCT3/4-shp53-F, pCXLE-hUL and pCXLE-hSK Okita et al. (2011). After electroporation, cells were kept in fibroblast medium for three days, then for the rest of the reprogramming in ESC medium consisting of KnockOut DMEM (Gibco) supplemented with 20% KnockOut Serum Replacement, 1% non-essential amino acids (Gibco), 1 mM L-Glutamine (Gibco), 0.1 mM β-mercaptoethanol (Gibco), 4 ng/ml human recombinant bFGF (R&D Systems). Medium was changed daily until iPSC colonies formed. After manual picking, iPSC lines were maintained on Matrigel (Corning) coated plates in mTeSR1 medium (Stem Cell Technologies).

3.2. RT-PCR analysis

RNA was extracted using Trizol® (Invitrogen), 1 μg RNA was digested with DNAseI (Invitrogen) according to the manufacturer's instructions and cDNA was synthesized from 0.5 μg DNAseI digested RNA using SuperScript® III Reverse Transcriptase and Oligo$(dT)_{20}$ primer (both Invitrogen) according to the manufacturer's protocol. PCR reactions were performed using Crimson Taq Polymerase (New England Biolabs) using standard PCR conditions (95 °C 30 s (1×); 95 °C 30 s, 55–66 °C 1 min, 68 °C 1 min (35×); 68 °C 5 min (1×), 12 °C (hold); for details see Table 1). PCR products were subjected to agarose gel electrophoresis.

3.3. In vitro differentiation via embryoid body formation

iPSCs were harvested using ReLeSR (Stem Cell Technologies), singled, counted and 9×10^5 cells per well were transferred to an AggreWell 800™ plate (Stem Cell Technologies) containing ESC medium without bFGF but supplemented with 10 μM ROCK inhibitor (Y-27632; SIGMA) to form uniform embryoid bodies (EBs) overnight. The following day, EBs were transferred to Ultra low attachment plates (Greiner) and cultivated for 6 days in ESC medium without bFGF. On day 8, EBs were dissociated with Trypsin (Invitrogen) and plated onto gelatin coated plates (with cover slips for immunofluorescence staining, without for RNA isolation and RT-PCR). Medium was switched to differentiation medium (ESC medium without bFGF/MEF medium 1:1 (*v*/*v*)) and cells were cultivated for 7 more days. Cells were fixed on day 15 of differentiation with 4% paraformaldehyde (PFA) in PBS for immunofluorescence staining or lysed with Trizol® for RNA isolation and RT-PCR.

Table 2
Antibodies used for immunofluorescence staining.

Application	Antibody	Dilution	Company	Cat. #
Pluripotency	Mouse anti-Oct4	1:100	Santa Cruz	sc-5279
	Mouse anti-Sox2	1:100	Merck Millipore	MAB4343
	Mouse anti-Nanog	1:100	Thermo Fisher Scientific	MA1-017
	Mouse anti-SSEA4	1:100	Santa Cruz	sc-21,704
	Mouse anti-AFP	1:100	Abcam	ab3980
Differentiation	Rabbit anti-SMA	1:100	Abcam	ab5694
	Mouse anti-βIII-Tubulin	1:100	Abcam	ab78078
Secondary antibodies	Alexa Fluor goat anti mouse 488	1:500	Thermo Fisher Scientific	A-10667
	Goat anti-Rabbit IgG (H + L), TRITC conjugate	1:500	Thermo Fisher Scientific	A16101

3.4. Immunofluorescence staining

Presence of specific pluripotency marker genes (Oct3/4, Sox2, Nanog, SSEA-4) or germ layer markers (AFP, βIII-Tubulin, SMA) was analysed using immunofluorescence staining. For detection of pluripotency markers, iPSCs were seeded onto gelatine-coated cover slips in Essential 8™ Flex medium (Gibco) and cultivated for 4 days before fixation. iPSCs or differentiated cells after EB formation were fixed with 4% PFA/PBS (15 min, room temperature (RT)), permeabilized with 1% Triton X-100/PBS for 10 min at RT and blocked with 5% BSA/0.1% Triton X-100/PBS for 1 h at RT. Cells were incubated with the primary antibodies (Table 2) in 5% BSA/PBS for 1 h at RT, followed by incubation in 5% BSA/PBS with the secondary antibodies (Table 2) for 30 min at RT. Nuclei were stained with DAPI included in the mounting medium (Fluoromount-G with DAPI, eBioscience). Cells were analysed using a fluorescent microscope (Leica) using Leica Imaging Software.

3.5. Karyotyping

After trypsinisation, metaphases were prepared according to standard procedures. Fluorescence R-banding using chromomycin A3 and methyl green was performed as described in detail earlier (Schlegelberger et al., 1999). A minimum of 20 metaphase spreads were analysed and the chromosomes were classified according to the International System for Human Cytogenetic Nomenclature (ISCN 2013).

3.6. DNA isolation and Sanger sequencing

To confirm the presence of the pathogenic deletion in the TH gene, primers were designed to amplify the region of interest. iPSCs were lysed using DNAzol® (MRC Inc.) and DNA was extracted according to the manufacturer's instructions. 100 ng DNA was used for PCR using Crimson Taq Polymerase and standard PCR conditions (Table 1). PCR products were cloned into pGEM®-TEasy (Promega) and 10 clones for each mutation where sequenced by GATC Biotech (Köln, Germany).

Acknowledgements

pCXLE-hOCT3/4-shp53-F, pCXLE-hSK and pCXLE-hUL were kind gifts from Shinya Yamanaka (Addgene plasmids #27077, #27078, and #27080). This work was supported by the Deutsche Forschungsgemeinschaft (DFG, German Research Foundation) for the Cluster of Excellence REBIRTH (From Regenerative Biology to Reconstructive Therapy; EXC 62/3 to G.G.) and in part by the FP7-HEALTH-2012-INNOVATION-1 EU Grant No. 305444 (to N.B.).

References

Haase, A., Olmer, R., Schwanke, K., Wunderlich, S., Merkert, S., Hess, C., Zweigerdt, R., Gruh, I., Meyer, J., Wagner, S., Maier, L.S., Han, D.W., Glage, S., Miller, K., Fischer, P., Schöler, H.R., Martin, U., 2009. Generation of induced pluripotent stem cells from human cord blood. Cell Stem Cell 5 (4):434–441. http://dx.doi.org/10.1016/j.stem.2009.08.021.

Okita, K., Matsumura, Y., Sato, Y., Okada, A., Morizane, A., Okamoto, S., Hong, H., Nakagawa, M., Tanabe, K., Tezuka, K., Shibata, T., Kunisada, T., Takahashi, M., Takahashi, J., Saji, H., Yamanaka, S., 2011. A more efficient method to generate integration-free human iPS cells. Nat. Methods 8 (5):409–412. http://dx.doi.org/10.1038/nmeth.1591.

Schlegelberger, B., Metzke, S., Harder, S., Zühlke-Jenisch, R., Zhang, Y., Siebert, R., 1999. Diagnostic Cytogenetics. Springer.

Shaffer L.G. (Spokane, Wash.), McGowan-Jordan J. (Ottawa, Ont.), Schmid M. (Würzburg) (Editors), ISCN 2013. An International System for Human Cytogenetic Nomenclature (2013). Recommendations of the International Standing Committee on Human Cytogenetic Nomenclature Published in collaboration with 'Cytogenetic and Genome Research' plus fold-out: 'The Normal Human Karyotype G- and R-bands'. Karger, 2013, ISBN: 978-3-318-0225.